Chemical Imaging of Single Apoptotic Cells using Fluorescence-Assisted Infrared Micro-Spectroscopy

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Using synchrotron infrared microspectroscopy, Jamin *et al.* have first demonstrated the potentiality of this technique in imaging the distribution of various chemical compounds in single living cells: chemical mapping of nucleic acids, proteins, and lipids in single living mouse hybridoma B cells at a resolution of 3 μm has been achieved (1). We have extended our preliminary investigation in trying to follow the evolution of the chemical distributions inside individual cells during one major cellular process: apoptosis .For such a purpose, we used mouse hybridoma B cell line, as for our initial experiments (1). Apoptosis was induced by using an anti-Fas monoclonal antibody (mAb) and this Fas positive hybridoma B cell line. It allows us to control the kinetics of the apoptosis, and therefore is easily adaptable for any constraints imposed by the IR data recording procedure. The early and late stages of apoptosis were identified by using fluorescent markers: (a) annexin V (green label) binds to phosphatidyl serine residues in the cell membrane in early apoptosis, and (b) propidium iodide (orange label) intercalates into DNA in late stages of apoptosis, once the cell membrane has broken down. We induce apoptosis with the anti-Fas mAb, add annexin V and propidium iodide, and then collect cells at different evolving times. Using the new Continuμm IR microscope at U10B, which is the first IR microscope modified for simultaneous fluorescence microscopy capabilities, we are able to view healthy cells (colorless), cells in early apoptosis (green), cells in late apoptosis (yellow=green+orange), and necrotic cells (orange).

Figure 1 displays the optical image of three cells 60 minutes after apoptosis induction. One microliter of cells was pipetted onto a diamond window and single cells were mapped with a 3x3 micron square aperture, and a 3-micron step size. **Figure 2** shows single spectra of a colorless, green, and yellow cell. Clear changes are observed in the phosphate (P=O) stretching region after induction of apoptosis. It is known that apoptosis involves numerous protein phosphorylation events and also dramatic changes in the nuclear material of the cell, both of which could yield changes in the P=O stretch region. The detailed analysis of the spectroscopic significance of these changes is underway. It should be noted that changes in the Amide I protein band are not observed in these spectra. This result is different than the process of necrosis, where a C=O ester group arises as protein oxidation occurs. These unique differences between apoptosis and necrosis may provide important markers for studying cell death in the future.

References: N. Jamin, P. Dumas, J. Montcuit, W.H. Fridman, J.L. Teillaud, G.L. Carr, and G.P. Williams, *Proc. of the National Acad. Sci.*, vol. 95, pp. 4837, 1998.

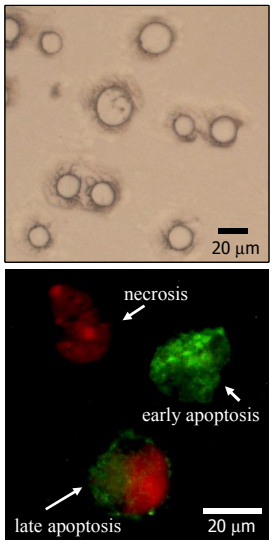

Figure 1. (Top) Apoptotic mouse hybridoma B cells under normal illumination, 320x magnification. (Bottom) Apoptotic mouse hybridoma B cells under fluorescent illumination (400x).

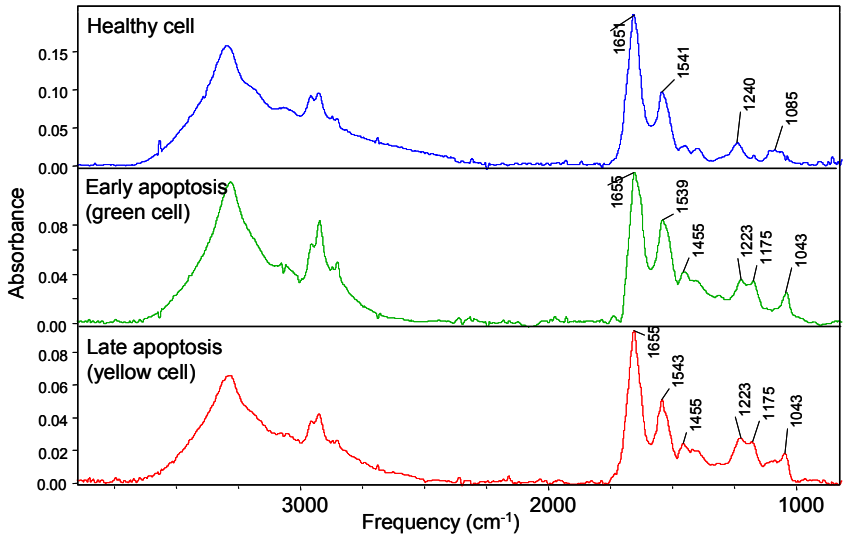

Figure 2. Infrared spectra from the center (10x10 micron aperture) of a healthy cell, an early apoptotic cell, and a late apoptotic cell. Clear changes are observed in the P=O stretching region (1200-1000 cm^{-1}).